Remarks

Claims 45, 46, 47, 48, 49, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 and 75 are pending. Claims 47, 50 and 51 have been newly cancelled. Claims 45, 46, 47, 48, 49, 52 and 53 have been newly amended. Claims 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74 and 75 are newly added. Support for these amendments are found throughout the specification and in the claims as originally filed. No new matter has been entered. All newly added claims are encompassed by Group I of the restriction requirement drawn to methods of identifying biomarkers and methods for diagnosis and prognosis of coronary artery disease, further restricted to the ABCA1 gene.

Claims 67, 68, 69, 70 and 73 clarify that said levels of RNA encoded by said gene are in blood samples leukocytes which include all of the types of leukocytes in whole blood, i.e. of blood samples which include granulocytes in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes). This phrase finds clear support in the specification, including at Figure 5C which shows standardized levels of insulin gene in each of the fractions of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes (G.R.), lymphocytes [T-lymphocytes (CD 3+) and B-lymphocytes (CD19+)] and monocytes (MONO) represent all of the types of leukocytes found in blood is taught at Fig. A.23 Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. (attached) which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. Additional support for the term "leukocytes" is found at paragraphs [0003] and [0085] of US20060134635 (interchangeably referred to herein as "the published application").

New independent claim 75 claims a method of classifying gene expression of an ABCA1 gene in a test subject relative to a population of control subjects that includes subjects having coronary artery disease and healthy subjects. New claim 75 comprises a step of quantifying a level of RNA encoded by the gene in a blood sample of the test subject, and a subsequent step of comparing the level in the sample of the test subject with level of RNA encoded by the gene in blood samples of the subjects having coronary artery disease and in blood samples of the healthy subjects. The new claim concludes that a determination that the level in the sample of the test subject is statistically similar to the level in the samples of the subjects having coronary artery disease and is statistically lower than the level in the samples of the healthy subjects classifies the level in the sample of the test subject with the level in the samples of the subjects having coronary artery disease; and that a determination that the level in the sample of the test subject is statistically higher than the levels in the samples of the subjects having coronary artery disease and is statistically similar to the levels in the samples of the healthy subjects classifies the level in the sample of the test subject with the levels in the samples of the healthy subjects. Support for reciting comparison of biomarker RNA levels of a test subject with those of control subjects having a disease (i.e. coronary artery disease) and with those of healthy control subjects, and determination of a statistically significant similarity or difference therebetween can be found in the published application, for example at paragraph [0119] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"), at paragraph [0120] ("*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*"). Support for reciting classification of a test subject level with specific control levels can be found, for example, at claim 12 as originally filed ("*d) determining whether the level of said one or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with the levels of said transcripts in step c)*"), at paragraph [0126] (relating to "*Methods that can be used for class prediction analysis*"), [0313] ("*Blood samples were taken from patients who were diagnosed with coronary artery disease as defined herein. Gene expression profiles were then analyzed and compared to profiles from patients unaffected by any disease.*").

Claims Rejection - 35 U.S.C. 112 2nd

Claims 47, 48, 49, 50, 51, 52 and 53 are rejected under 35 U.S.C. 112, 2nd paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The office action indicates that the recitation of "unfractionated samples of lysed blood" is unclear. Although Applicant respectfully traverses, Applicant has canceled independent claim 47 and dependent claims 50 and 51 solely for the purposes of advancing prosecution without prejudice for pursuing the unclaimed subject material in another application, rendering the rejection of claims 47, 50 and 51 moot. Applicant has amended dependent claims 48, 49, 52 and 53 to be dependent from claim 45 or newly added claim 56, which do not recite the phrase "unfractionated samples of lysed blood".

Claims Rejection - 35 U.S.C. 112 1st

Claims 47, 48, 49, 50, 51, 52 and 53 are rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the written description requirement on the grounds that the instantly recited phrase "unfractionated samples of lysed blood" is new matter. Although Applicant respectfully traverses, Applicant has canceled claim 47 and dependent claims 50 and 51 solely for the purposes of advancing prosecution without prejudice for pursuing the unclaimed subject material in another application, rendering the rejection of claims 47, 50 and 51 moot. Applicant has amended dependent claims 48, 49, 52 and 53 to be dependent from claim 45 or newly added claim 56, which do not recite the phrase "unfractionated samples of lysed blood".

Claims 45, 46, 47, 48, 49, 50, 51, 52 and 53 are rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the enablement requirement.

Applicant respectfully traverses. Applicant disagrees with the rejection's assertion that the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention in view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art.

The rejected claims include the steps of determining the level of RNA encoded by an ABCA1 gene in a blood sample obtained from a human test subject and comparing it to the level of control RNA encoded by the ABCA1 gene in blood samples of control subjects, wherein the comparison is indicative of coronary artery disease in said human test subject.

Applicant specifically traverses the statement on page 4 of the office action that "the independent claim, as written, states that a comparison of a human test subject ABCA1 RNA level in a blood sample to a control indicates that coronary artery disease is present in the test subject", and the statement on page 5 of the office action that the "claims are extremely broad because they set forth that any or all comparison between a test subject and RNA level from "control subjects" is indicative of disease". Applicant clarifies that the phrase "wherein said comparison of said quantified level of step (a) with said quantified level of said control subjects is indicative of coronary artery disease in said human test subject" of independent claim 45, is a narrowing limitation, limiting the claim to only those comparisons which are indicative of the test subject having coronary artery disease, and excluding those comparisons which do not indicate that the test individual has coronary artery disease.

However, in the interest of expediting prosecution, Applicant has added new claims which more clearly reflect the intention of the newly cancelled claims. Specific points raised in the instant enablement rejection will be addressed to the extent they are relevant to the newly added claims.

The rejection asserts that the claims are broad with respect to "control subjects", indicating that "control subjects" could encompass patients with coronary artery disease, healthy patients, and patients with some other disease such as Chagas disease, schizophrenia or a particular stage of coronary artery disease (page 5 of the office action). The instant claims recite two clearly defined sets of controls; patients having coronary artery disease and healthy controls. At least one claim, claim 59, limits the controls to healthy subjects.

The rejection asserts that the claims are very broad in scope because they encompass that any level and direction of difference in gene expression between the tested subjects is indicative of disease, (page 5 of the office action). As described above, Applicant disagrees with this claim interpretation. Accordingly, Applicant has newly added claims which specify a direction and a level of difference in ABCA1 expression required to be detected between the blood samples of

the test subject and the healthy controls. For example, claim 71 recites "wherein said test subject is a candidate for having if the level of RNA encoded by said ABCA1 gene in said blood sample of said human test subject is at least 1.5 times *lower* than that of said healthy subjects with a p value less than 0.05". Such a statistical probability will not likely be achieved comparing one test subject with only two control subjects, thereby addressing the concern raised in the instant rejection over the minimum number of controls necessary for a meaningful comparison at pages 6 and 9 of the instant office action.

By reciting that the controls are healthy subjects, newly added claim 71 also addresses the issue raised in the instant rejection concerning detecting coronary artery disease in a test subject based on a comparison between the test individual and control individuals where the control individuals don't have coronary artery disease, but could still have some other disease or condition, as suggested at pages 5 and 8 of the office action. Applicant respectfully traverses the assertion at page 7 of the office action that "there is no guidance or analysis of data in the specification to suggest that this gene in particular is sufficient to conclude that coronary artery disease is present". Applicant submits that this gene is indeed sufficient to provide an indication of coronary artery disease on the grounds that the specification discloses that RNA encoded by the ABCA1 gene in a blood sample of from a coronary artery disease patient is differently expressed relative to healthy subjects with a p value less than 0.05, see Example 21, Figure 19 in which patients with coronary artery disease and healthy control individuals were analyzed. In addition, Applicant's position is clearly supported by the attached declaration filed under 1.132 which discloses post-filing validation experiments using both quantitative RT-PCR (QRT-PCR), an alternate technology relative to microarray analysis employed in the experiments disclosed at Example 21 of the specification, and using an independent cohort of 14 healthy control subjects and 19 subjects having coronary artery disease relative to the subjects employed in the experiments disclosed at Example 21 of the specification. The experiments disclosed in the declaration clearly show that RNA encoded by the gene ABCA1 is present at statistically lower levels in blood of subjects having coronary artery disease relative to healthy control subjects.

The rejection also asserts that the specification does not establish any particular level of expression of ABCA1 which is sufficient to detect coronary artery disease to the exclusion of other disorders, at page 8 of the office action.

Solely for the purpose of expediting prosecution, Applicant has amended the claims to address this issue, for example, by including the limitation in claim 71 that the recited comparison between a test subject and controls indicates that the test individual is a "candidate" for having coronary artery disease, and by adding claim 75 which merely claims a method of classifying the expression level of ABCA1 in blood of a test subject with that of healthy subjects or subjects having coronary artery disease.

The office action indicates that it would take undue experimentation to practice the invention, specifically to determine difference thresholds required to determine that a patient has or does not have disease, pages 9 and 11 of the office action, and that the invention is in an area that is highly unpredictable, page 11 and throughout the office action. Applicant respectfully disagrees. MPEP 2164.03 indicates that "the "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art." Because of the guidance in the specification which shows a statistically significant correlation between the levels of ABCA1 RNA in blood of diseased vs. healthy controls, Applicant contends that one of skill can reasonably predict with a statistically significant probability that a patient may be a candidate for having coronary artery disease based on the teachings in the specification.

The office action specifically contends in an attempt to demonstrate the unpredictability of the instant claims that Albrecht et al. (Albrecht et al., 2004. Stroke 35:2801) did not observe a difference in expression of ABCA1 gene in blood cells from patients having carotid artherssclerotic plaques (that is coronary artery disease) versus healthy patients, thereby failing to replicate the results disclosed in the specification.

Applicant respectfully disagrees, particularly on the grounds that the maximum detection sensitivity achieved by Albrecht *et al.*, and concomitant minimum fold-change criterion employed in Albrecht *et al.* to determine whether ABCA1 is differentially expressed do not enable detection of differential expression of ABCA1. Namely, Albrecht *et al.* teaches that the criterion for a significant fold-change is $\geq$2-fold differential expression (page 2804, 1st paragraph). However, the experiments disclosed in the declaration clearly show that this gene is present at 1.5-fold lower levels in blood of coronary artery disease patients relative to healthy

subjects, which is below the minimum >2-fold threshold set by Albrecht *et al.* for detection of differential expression. Applicant submits that the failure of Albrecht *et al.* to report differential expression therefore inherently cannot support the contention that the claimed invention is not enabled on grounds of unpredictability.

The office action states that Lee teaches that data obtained from microarrays must be replicated in order to screen out false positive results; that Cheung et al. (2003) teaches that there is natural variation in gene expression amongst different individuals; that Wu et al (2001) teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, and that the conclusions that can be drawn from a given set of data depend on the particular choice of data analysis; and Newton et al. (2001) teaches that a replication of data is required for validation.

While disagreeing with the office action's contention based on Newton *et al.* that data replication would be necessary to enable the claims, the attached declaration filed under 1.132 discloses post-filing validation experiments using quantitative RT-PCR (QRT-PCR), an alternate technology relative to microarray analysis employed in the experiments disclosed at Example 21 of the specification, as well as using an independent cohort of control and disease subjects relative to those employed in the experiments disclosed at Example 21 of the specification. The experiments disclosed in the declaration clearly show that RNA encoded by the gene ABCA1 is present at statistically lower levels in blood of subjects having coronary artery disease relative to healthy control subjects. The experimental data disclosed in the declaration clearly addresses the office action's concerns that replication of experiments may be required and that the results disclosed in the specification are intrinsic to the particular cohort used.

Applicant respectfully disagrees with the contention in Wu et al. that expression data needs to be interpreted in view of other biological knowledge. Differential gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly from the state of disease of the individual. Rather these changes in expression can be as a result of a downstream effect of pathogenic processes, and it is not necessary that the biological relevance of the data be known to allow this difference in expression to be useful as a biomarker. For example prostate-specific phosphatase and prostate-specific antigen (PSA) were long used as biomarkers without an understanding of their function

(refer, for example, to the enclosed abstracts of: Chu TM, 1990, Prostate cancer-associated markers. Immunol. Ser. 53:339-56; and Diamandis EP., 2000, Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900).

The Examiner also argues, on the basis of post-filing art of Wu (2001) and Newton (2001), that many factors may influence the outcome of the data analysis and notes that conclusions depend on the methods of data analysis. While considerations such as variability, and normalization are of importance, these considerations are well understood by a person skilled in the art and have been applied for many years to permit development of biomarkers which are indicative of disease. These challenges are well understood, as are the routine experiments required to exemplify statistically significant differences in populations.

Applicant notes that the results disclosed by Cheung *et al.* cannot be reliably extrapolated to primary blood samples since the lymphoblastoid cells employed by Cheung *et al.* are significantly modified relative to primary blood cells, due to being cultured cell lines generated by immortalization of primary human cells derived from "CEPH" families, as indicated in Reference no. 10 of Cheung *et al.* (Dausset *et al.*, 1990. Genomics 6:575; enclosed) at p. 575, right column, 1st paragraph. Applicant notes that immortalized cultured cell lines such as the lymphoblastoid cells taught by Cheung *et al.* undergo significant genetic modification such as strong genome-wide demethylation (refer, for example, to enclosed abstract of: Vilain *et al.*, 2003. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93), as a result of extensive *in-vitro* culturing in the absence of immune or apoptotic mechanisms which function to eliminate mutated cells in the body. As such, immortalized CEPH lymphoblastoid cells may represent a particularly unsuitable cell type for modeling gene expression variability in primary blood cells.

To the extent that Cheung et al. could still be considered to suggest that larger populations of diseased and control populations may be useful to determine what level of differential expression is indicative of disease amongst the population at large, the Applicant submits that the extension of the experiments as outlined in the specification to additional individuals is merely routine. As is noted in Re Wands "*even a considerable amount of experimentation is permissible to practice the claimed methods, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction*

in which the experimentation should proceed.'" (Re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)).

Furthermore, the decision *In re Angstadt*, 190 U.S.P.Q. 218 (C.C.P.A. 1976) clearly states that even in an unpredictable art, and clearly permits the presence of a screening step to identify those embodiments which possess the desired activity is permissible. In fact, in *Angstadt*, the Court specifically dismissed the notion that the specification must provide a level of guidance that would predict the outcome of an experiment "with reasonable certainty before performing the reaction" and that "such a proposition is contrary to the basic policy of the Patent Act, which is to encourage disclosure of inventions and thereby to promote progress in the useful arts." The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention.

Applicant wishes to point out that in *In re Wands*, the court stated that "[e]nablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue' not 'experimentation' (citing *In re Angstadt*, 537 F. 2d 498 at 504, 190 U.S.P.Q. 214 at 219 (C.C.P.A. 1976)). The Court also stated that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." (citing In re Jackson, 217 U.S.P.Q. 804 at 807 (Bd. App. 1982)).

As such the Applicants believe there is sufficient guidance provided by the specification and that the art is sufficiently predictable such that the amount of experimentation to perform the subject matter within the instant claims is not undue.

In light of the amendments and above remarks, the Applicant contends that the claims are fully enabled, and respectfully requests reconsideration and withdrawal of the instant rejections.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. No new matter is added. If the Examiner believes that a

telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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